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Associations Between Respiratory Arrhythmia and Fundamental Frequency of Spontaneous Crying in Preterm and Term Infants at Term-Equivalent Age

ABSTRACT: *This study investigated whether lower vagal function in preterm infants is associated with increased fundamental frequency (F_0; frequency of vocal fold vibration) of their spontaneous cries. We assessed respiratory sinus arrhythmia (RSA) during quiet sleep as a measure of vagal function, and its relationship with the F_0 of spontaneous cries in healthy preterm and term infants at term-equivalent age. The results showed that preterm infants have significantly lower RSA, and higher overall F_0 than term infants. Moreover, lower RSA was associated with higher overall F_0 in preterm infants, whereas higher RSA was positively associated with mean and maximum F_0, and a larger F_0 range in term infants. These results suggest that individual differences in vagal function may be associated with the F_0 of spontaneous cries via modulation of vocal fold tension in infants at an early developmental stage.* © *2016 The Authors. Developmental Psychobiology Published by Wiley Periodicals, Inc.* Dev Psychobiol 9999: 1–10, 2016.

Keywords: *heart rate variability; respiratory sinus arrhythmia; vagal tone; cardiac vagal index; autonomic nervous system; crying; fundamental frequency (F_0); preterm infants; low-birth-weight infants*

INTRODUCTION

Acoustic features of infant cries have been researched as a non-invasive tool for assessing neurophysiological states, for example, in the context of assessing pain stress (Johnston, Stevens, Craig, & Grunau, 1993; Porter, Porges, & Marshall, 1988; Stevens et al., 2007) and medical complications (Michelsson & Michelsson, 1999; Soltis, 2004; Wasz-Höckert, Michelsson, & Lind, 1985). In particular, the fundamental frequency (F_0; the vibration frequency of the vocal folds, generally perceived as pitch) of cries has been reported to be abnormally high (e.g., mean $F_0 > 600$ Hz) in infants with several medical complications, such as chromosomal, endocrine, metabolic, and neurological disturbances, at an early developmental stage (Soltis, 2004). Preterm birth is also a factor in the higher F_0 of cries

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during early infancy. Several previous studies have reported that, compared to term newborns, the F_0 of cries of preterm infants are higher before term-equivalent age (Johnston et al., 1993; Michelsson, Järvenpää, & Rinne, 1983) or even at term-equivalent age (Shinya, Kawai, Niwa, & Myowa-Yamakoshi, 2014). However, it remains unclear why preterm infants' cries possess a higher F_0 during early infancy, although some possible factors have been discussed.

Preterm infants typically have smaller body sizes before term-equivalent age than term newborns. Higher vocal F_0 is generally associated with smaller body size (particularly, vocal fold lengths) among typically developing infants and children, although F_0 also depends on a complex interaction between laryngeal and respiratory controls (Titze & Ingo, 1994). Thus, the higher F_0 of preterm infants could be assumed to solely reflect premature body development. Indeed, the F_0 of pain-induced cries in preterm infants around term-equivalent age has been reported to be similar to term newborns (Cacace, Robb, Saxman, Risemberg, & Koltai, 1995; Johnston et al., 1993; Michelsson et al., 1983), probably as the body size of preterm infants catches up to near the level of term infants. However, we recently demonstrated that the F_0 of spontaneous cries (i.e., those unaffected by external acute stress) in preterm infants is still higher at term-equivalent age than it is in term infants (Shinya et al., 2014). Furthermore, a higher F_0 was also related to shorter gestational age, regardless of body size (e.g., weight, height, head and chest circumference). These findings suggest that the higher F_0 of spontaneous cries in preterm infants may be caused by more complex neurophysiological conditions associated with their different intrauterine and extrauterine experiences.

Another possible factor in the higher F_0 in spontaneous cries is the lower function of the autonomic nervous system in preterm infants (Michelsson et al., 1983; Shinya et al., 2014). In the literature on infant crying, the role of the vagus, the tenth cranial nerve of the parasympathetic nervous system, has been emphasized as the most proximate neural input affecting F_0 (Golub & Corwin, 1985; Porges et al., 1994; Porter et al., 1988; Soltis, 2004). Increases in vocal F_0 are predominantly modulated by vocal fold tension, due to contraction of the intrinsic laryngeal muscles, innervated by sympathetic and parasympathetic (vagal) input from the autonomic nervous system. More specifically, vagal input from the right nucleus ambiguus of the medulla has an inhibitory effect on the contraction of the laryngeal muscles. Thus, diminished vagal activity in response to acute stress is assumed to cause laryngeal muscle contraction and tightening of the vocal folds, resulting in a higher F_0 of infant crying (Porter et al., 1988).

In fact, previous studies have reported that preterm infants have a higher heart rate and a lower heart rate variability (HRV) than term infants (De Rogalski Landrot et al., 2007; Eiselt et al., 1993; Fyfe et al., 2015; Longin, Gerstner, Schaible, Lenz, & König, 2006; Patural et al., 2008; Yiallourou, Witcombe, Sands, Walker, & Horne, 2013). In particular, the most significant difference was in the high frequency component of HRV (i.e., respiratory sinus arrhythmia [RSA]), which reflects vagal (parasympathetic) modulation on the heart. RSA in preterm infants increases gradually with postmenstrual age from birth to the term period (Feldman, 2006; Khattak et al., 2007; Krueger, van Oostrom, & Shuster, 2009; Padhye, Williams, Khattak, & Lasky, 2009; Sahni et al., 2000); nevertheless, it is still lower than term infants even at a term-equivalent age (De Rogalski Landrot et al., 2007; Eiselt et al., 1993; Patural et al., 2004, 2008) or early infancy (Fyfe et al., 2015; Yiallourou et al., 2013). Thus, it is possible that the lower vagal function may be associated with the higher F_0 of spontaneous cries in preterm infants at term-equivalent age.

However, very few studies have investigated the relationship between vagal function and the F_0 of infant crying, even in typically developing infants. To the best of our knowledge, only two studies have investigated the issue. Porter et al. (1988) reported the relationship between cardiac vagal tone and the F_0 of crying in term newborns experiencing a circumcision procedure; vagal tone was significantly reduced during the severely stressful procedure, and the reduction was paralleled by a significant increase in the F_0 of the infants' cries. In the other study, Stewart et al. (2013) reported co-variation between autonomic states and acoustic features of cries in 6-month-old infants experiencing the still-face paradigm. They demonstrated that the reduction in RSA following the paradigm was associated with decreased modulation of acoustic features (e.g., less variation in F_0). These findings are consistent with the assumption that the myelinated branch of the vagus, originating in the same brainstem structures, is involved in both the regulation of heart rate and laryngeal muscles (Ayres & Gabbott, 2002; Bieger & Hopkins, 1987; Porges, 2007), suggesting that vagal influence on the heart may reflect vagal output to the laryngeal muscles, related to the F_0 of infant crying.

Furthermore, it is still unclear how individual differences in vagal function are related to the F_0 of infant crying. Porter et al. (1988) also reported that lower resting vagal tone was significantly associated with a higher F_0 of pain-induced cries as well as smaller changes in both vagal tone and heart period during surgery. This suggests that individual differences in vagal function may predict stress responses, including the F_0 of infant crying. However, whether the low

vagal function in preterm infants is related to the increased F_0 of crying remains to be investigated. Considering that RSA is lower in preterm infants than in term infants at term-equivalent age, the higher F_0 of spontaneous cries in preterm infants may reflect hypertension of vocal folds induced by lower vagal function rather than their smaller body size.

Here, we expand on the previous observations between autonomic states and acoustic features of infant crying in preterm infants. We investigated the relationships between RSA during quiet sleep and the F_0 of spontaneous cries before feeding in preterm and term infants at term-equivalent age. Based on a previous neural blockade study, the vagal (parasympathetic) influence on the heart is assumed to reflect the high-frequency component of heart rate variability (HRV), accompanied by the respiratory cycle (i.e., RSA); however, RSA may not solely reflect tonic vagal control of the heart rate when the respiratory rate varies during activities that affect central respiratory drive (Houtveen, Rietveld, & Geus, 2002; Task Force, 1996). Thus, this study focused on RSA during quiet sleep to assess individual differences in vagal function. We hypothesized that lower RSA would be significantly related to a higher F_0 of spontaneous cries in preterm infants at term-equivalent age.

MATERIALS AND METHODS

Participants

Thirty healthy preterm infants (gestational age [GA] <37 weeks) and 30 term controls (GA ≥ 37 weeks) participated in this study at term-equivalent age (i.e., postmenstrual age between 37 and <42 weeks). All participants were recruited between 2013 and 2015 from the neonatal intensive care unit at Kyoto University Hospital, Japan. Inclusion criteria included that the subjects had no severe neurological complications, such as brain lesions (including periventricular leukomalacia, Grade III or IV intraventricular hemorrhages) or chromosomal abnormalities, and that they did not require medical treatment for respiratory disease during our recordings. In all, 10 infants were excluded because of shorter recording during quiet sleep (<5 min) due to consistent arousal or active sleep, leading to a total 25 preterm (GA, mean [SD] $= 32.0 \pm 3.2$ weeks, range $= 25.6$–36.0) and 25 term (GA, mean [SD] $= 39.5 \pm 1.1$ weeks, range $= 37.1$–41.4) infants. Table 1 shows the demographic data for the participants.

Procedures

The study was conducted with the approval of the ethics committee of Kyoto University Graduate School and Faculty of Medicine (No. E581). Written informed consent was obtained from the participants' parents. Infants were studied between five and nine p.m. while in a supine position in an open crib. Preterm infants were recorded in a growing care unit, where they stayed until leaving the hospital, while term infants were recorded in a quiet examination room at the hospital. Environmental conditions, including the crib used, the noise level, and ambient temperatures, were controlled for all participants. The noise level in the rooms was judged perceptually as low environmental noise, and was acceptable for audio recording and analysis. Electrocardiograph (ECG) data were recorded 0.5–1.5 hr after feeding, at a sampling rate of 1000 Hz from a neonatal monitor (Philips Intellivue MP 30 for preterm infants; Atom Neonatal Monitor V-1200 for term infants) with a12-bit A/D converter (TUSB-0412ADSM-SZ; Turtle industry Corp., Tokyo, Japan). Infants were videotaped during the entire recording session to document their behavioral state. A quiet sleep state was defined based on the following criteria: eyes closed with no rapid eye movement, no limb movement, and regular breathing and heart rate (e.g., Patural et al., 2004; Prechtl, 1974). Following ECG recording, the spontaneous cries of each infant less than 30 min before feeding were recorded for 60 s using a wave recorder at a 44.1 kHz sampling rate and with 16 bit quantization. During recording, the distance between the microphone and the infant's mouth was approximately 15 cm (EDIROL R-09; Roland Corp., Los Angeles, CA).

Measurements

Physiological Measures. We assessed RSA through a power spectral analysis, using an autonomic nervous analysis program (Map 1060; Nihon Santeku, Osaka, Japan). In addition to RSA,

Table 1. Demographic Data for Preterm and Term Infants

	Preterm ($n=25$)			Term ($n=25$)					
	M	*SD*	Range	*M*	*SD*	Range	*t*	*d*	*p*
Gestational age (weeks)	32.0	3.2	25.6–36.0	39.5	1.1	37.1–41.4	−10.91	3.08	$<10^{-11}$
Weight at birth (g)	1533.8	508.8	618–2572	2906.3	357.6	2352–4110	−11.04	3.12	$<10^{-13}$
Apgar score at 5 min[a]	8.2	1.7	4–10	9.2	0.5	8–10	−3.08	0.87	0.005
Postnatal age (days)	52.6	25.8	15–103	3.8	1.0	3–7	9.47	2.68	$<10^{-8}$
Postmenstrual age (weeks)	39.6	1.2	37.3–41.9	40.0	1.0	38.0–41.9	−1.39	0.39	0.170
Weight at cry recording (g)	2647.4	379.1	2130–3530	2768.1	375.1	2222–4028	−1.13	0.32	0.263
Female		16/25			16/25				

[a]Apgar score at 5 min: for preterms, median (IQR) = 8 (8–9); for terms, median (IQR) = 9 (9–10).

we calculated other HRV variables to describe group differences in autonomic function. For each infant, the recorded ECG data during quiet sleep for 5 min were converted into R-wave intervals after manual artifact correction. The corrected R-wave intervals were converted into heart rate. Power spectral HRV analysis was performed using a fast Fourier transform applied to each segment with a Hanning window, and determined following two main oscillations: a low-frequency component (LF, 0.04–0.24 Hz), representing both sympathetic and parasympathetic activity, related to the baroreflex system (Denver, Reed, & Porges, 2007; Malik & Camm, 1993; Task Force, 1996); and a high-frequency component (HF, 0.25–1.50 Hz) representing vagal (parasympathetic) activity modulated by respiratory cycles (i.e., RSA) (Denver et al., 2007; Malik & Camm, 1993; Task Force, 1996). For the high-frequency component, the bandwidth was extended to 0.25–1.50 Hz from the adult standard of 0.15–0.40 Hz (Task Force, 1996), due to the higher speed of respiration in newborn infants (Fortrat, 2002). Both HRV components were calculated by summing power spectral density values over the bandwidth. In addition, the ratio of LF to HF was calculated as an indicator of sympathovagal balance (Burr, 2007). Then the LF, RSA (HF), and LF/HF ratio were natural log transformed to fit assumptions of linear analyses (for cardiac vagal index (Toichi, Sugiura, Murai, & Sengoku, 1997), see Supplement 1 in electronic supplementary materials).

Acoustic Measures. A cry utterance was defined as a vocal output occurring on a single expiration and lasting for at least 0.3 s to exclude non-cry sounds, such as coughs (Shinya et al., 2014). F_0 variables were assessed using Praat (ver. 5.4) (Boersma & Weenink, 2014). In total, 1,930 cries were extracted, and those containing broad regions of environmental noise were excluded from the analysis to avoid artifacts when determining the F_0. Ultimately, 1,670 cries (~86.1% of all cry utterances) were used in the acoustic analyses (preterm group, mean number of cries per infant = 36.2 [range: 13–71], term group, 30.6 [8–51]).

The cry utterances were down-sampled to 22.05 kHz and low-pass filtered at 10 kHz to eliminate outliers and artifacts; then, the minimum F_0 (the lowest fundamental frequency of a cry utterance), mean F_0 (the mean fundamental frequency), maximum F_0 (the highest fundamental frequency), and F_0 range (the difference between maximum and minimum F_0) were determined using a Praat autocorrelation algorithm: a noise-resistant autocorrelation method at 150–900 Hz with a Hanning window length of 0.05 s. Mean values of each F_0 measure were calculated for each infant and related to RSA.

The overall F_0 may be related to measures of body weight and height among normally developing infants (Titze & Ingo, 1994), as well as pathological conditions in the neonatal period (e.g., preterm birth, chromosomal abnormalities, bacterial meningitis, congenital hypothyroidism, herpes simplex viral encephalitis, hyperbilirubinemia, and hypoglycaemia (Soltis, 2004; Wasz-Höckert et al., 1985). Specifically, the minimum F_0 is assumed to be most affected by body size, corresponding to the larynx, particularly the vocal folds (Ey et al., 2007; Wermke & Robb, 2010). However, maximum F_0 is related to neurological control that coordinates respiratory-phonatory activity (Wermke, Mende, Manfredi, & Bruscaglioni, 2002), in addition to body indices (Wermke & Robb, 2010).

RESULTS

Comparisons of Physiological and Acoustic Variables in Preterm and Term Infants

Physiological and acoustic variables for each group are shown in Table 2. For physiological variables, an unpaired Student's *t*-test revealed that RSA was significantly lower in preterm infants than term infants ($t = -6.90$, $d = 1.95$, $p < 10^{-7}$). In addition, preterm infants had a higher heart rate ($t = 7.64$, $d = 2.16$, $p < 10^{-9}$) and LF/HF ($t = 3.31$, $d = 0.94$, $p < 0.002$), although they had lower LF ($t = -4.50$, $d = 1.27$, $p < 10^{-4}$), compared to term infants. These results indicate both lower vagal (parasympathetic) and sympathetic modulation of the heart, and higher sympathovagal balance during quiet sleep in preterm infants. For acoustic variables, the spontaneous cries of preterm infants had significantly higher minimum F_0 ($t = 2.62$, $d = 0.74$, $p = 0.012$), mean F_0 ($t = 2.96$, $d = 0.84$, $p = 0.005$), and maximum F_0 ($t = 3.21$, $d = 0.91$, $p = 0.002$) values. On the other hand, there were no significant differences in F_0 range between preterm and term infants ($t = 1.44$, $d = 0.41$, $p = 0.155$).

Relationships Between RSA and Crying F_0

To investigate the relationships between RSA and crying F_0, we performed Pearson's correlation analyses (Fig. 1). For all participants, RSA was significantly negatively correlated with minimum F_0 ($r = -0.41$, $p = 0.003$), mean F_0 ($r = -0.39$, $p = 0.005$), and maximum F_0 ($r = -0.36$, $p = 0.011$), but not with F_0 range ($r = -0.01$, $p = 0.957$). Similarly, in preterm infants, RSA was significantly negatively correlated with minimum F_0 ($r = -0.49$, $p = 0.014$), mean F_0 ($r = -0.46$, $p = 0.019$), and maximum F_0 ($r = -0.46$, $p = 0.022$), but not with F_0 range ($r = 0.02$, $p = 0.933$). However, in the term group, RSA was marginally significantly positively correlated with maximum F_0 ($r = 0.40$, $p = 0.051$) and F_0 range ($r = 0.36$, $p = 0.075$) (for other correlations between physiological and acoustic variables, see Supplement 2 and Table S1 in electronic supplementary materials).

To further analyze the independent contribution of vagal function to crying F_0, we performed hierarchical multiple regression analyses with the F_0 variables as dependent variables in the preterm and term groups. As independent variables, gestational age, postmenstrual age, and weight at cry recording were entered in the first step,

Table 2. Comparisons of Physiological Variables During Quiet Sleep and Acoustic Variables During Spontaneous Crying in Preterm and Term Infants

	Preterm ($n = 25$)			Term ($n = 25$)					
	M	*SD*	Range	*M*	*SD*	Range	*t*	*d*	*p*
Physiological variables									
Heart rate (bpm)	139	11	107–154	112	13	88–144	7.64	2.16	$<10^{-9}$
LF (ln ms^2)	2.12	1.12	−0.51 to 4.50	3.53	1.11	1.52–5.27	−4.50	1.27	$<10^{-4}$
RSA (HF) (ln ms^2)	0.95	1.22	−1.15 to 2.66	3.24	1.12	1.02–5.51	−6.90	1.95	$<10^{-7}$
LF/HF (ln)	1.18	0.87	−0.32 to 2.69	0.31	0.74	−1.04 to 1.90	3.83	1.08	<0.001
Acoustic variables									
Minimum F_0 (Hz)	348	61	266–507	310	38	245–389	2.62	0.74	0.012
Mean F_0 (Hz)	451	66	344–571	404	44	335–491	2.96	0.84	0.005
Maximum F_0 (Hz)	524	64	423–644	472	50	395–574	3.21	0.91	0.002
F_0 range (Hz)	176	33	112–250	162	39	88–276	1.44	0.41	0.155

Note: LF, low frequency component of HRV; ln ms^2, natural logarithm of the absolute values; RSA, respiratory sinus arrhythmia (i.e., high frequency component of HRV [HF]); LF/HF, natural logarithm of the ratio of LF to HF.

and RSA was entered in the second step. In this model, we excluded the other demographic variables related to gestational age (i.e., birth weight, Apgar score at 5 min, and postnatal age) to avoid collinearity of predictors (Supplement 3; Table S2). Postmenstrual age was also excluded from the model for term infants because there was a strong correlation between postmenstrual and gestational age in this group (Supplement 3; Table S2).

A summary of hierarchical regression analyses are shown in Tables 3 and 4. For the preterm group, although the first step (gestational age, postmenstrual age, and weight at cry recording) did not significantly predict any F_0 variable of spontaneous cries, step two resulted in significant values for minimum F_0 ($\Delta R^2 = 0.23$, $\Delta F_{4,20} = 6.93$, $p = 0.016$), mean F_0 ($\Delta R^2 = 0.19$, $\Delta F_{4,20} = 5.16$, $p = 0.034$) and maximum F_0 ($\Delta R^2 = 0.17$, $\Delta F_{4,20} = 4.61$, $p = 0.044$), indicating that lower RSA predicted higher minimum F_0 ($\beta = -0.51$, $t = -2.63$), mean F_0 ($\beta = -0.46$, $t = -2.27$), and maximum F_0 ($\beta = -0.45$, $t = -2.15$) (Table 3). For the term group, the first step also did not significantly predict any F_0 variable of spontaneous cries, whereas step two resulted in significant values for mean F_0 ($\Delta R^2 = 0.15$, $\Delta F_{3,21} = 4.60$, $p = 0.044$), maximum F_0 ($\Delta R^2 = 0.23$, $\Delta F_{3,21} = 7.08$, $p = 0.015$), and F_0 range ($\Delta R^2 = 0.20$, $\Delta F_{3,21} = 5.60$, $p = 0.028$), indicating that higher RSA predicted a higher mean F_0 ($\beta = 0.41$, $t = 2.14$), maximum F_0 ($\beta = 0.51$, $t = 2.66$), and larger F_0 range ($\beta = 0.47$, $t = 2.37$) (Table 4).

DISCUSSION

This study examined the associations between vagal function, indexed by RSA during quiet sleep, and F_0 of spontaneous cries in preterm and term infants at term-equivalent age. Lower RSA was associated with increased overall F_0 in preterm infants, consistent with our hypothesis. In contrast, higher RSA was associated with higher mean and maximum F_0, and F_0 range in term infants. These differences may be due to the robustly different levels of vagal function between preterm and term infants.

In line with previous studies (De Rogalski Landrot et al., 2007; Eiselt et al., 1993; Patural et al., 2008), we found that preterm infants had lower HRV parameters, particularly in the HF component (i.e., RSA), during quiet sleep than term infants at term-equivalent age, indicating that preterm infants showed markedly reduced vagal influence on the heart. Comparable with these results, their LF/HF ratios were also higher than term infants, reflecting a higher sympathetic predominance (for the associations between sympathovagal balance and crying F_0, see Supplement S4, Figure S1, and Table S3–4 in electronic supplementary materials). Moreover, we confirmed that the overall F_0 of spontaneous cries were significantly higher in preterm infants at term-equivalent age, consistent with the results in our previous report (Shinya et al., 2014).

As predicted, we found that lower RSA was significantly associated with a higher overall F_0 of spontaneous cries in preterm infants. Importantly, the associations still remained significant in hierarchical regression analyses, after adjusting for gestational age, postmenstrual age, and weight at cry recording, which potentially affect F_0 (Shinya et al., 2014). These results are consistent with a previous report that indicated that lower resting vagal tone was related to the higher F_0 of the pain-induced cries during a circumcision procedure (Porter et al., 1988). Decreased vagal activity from the

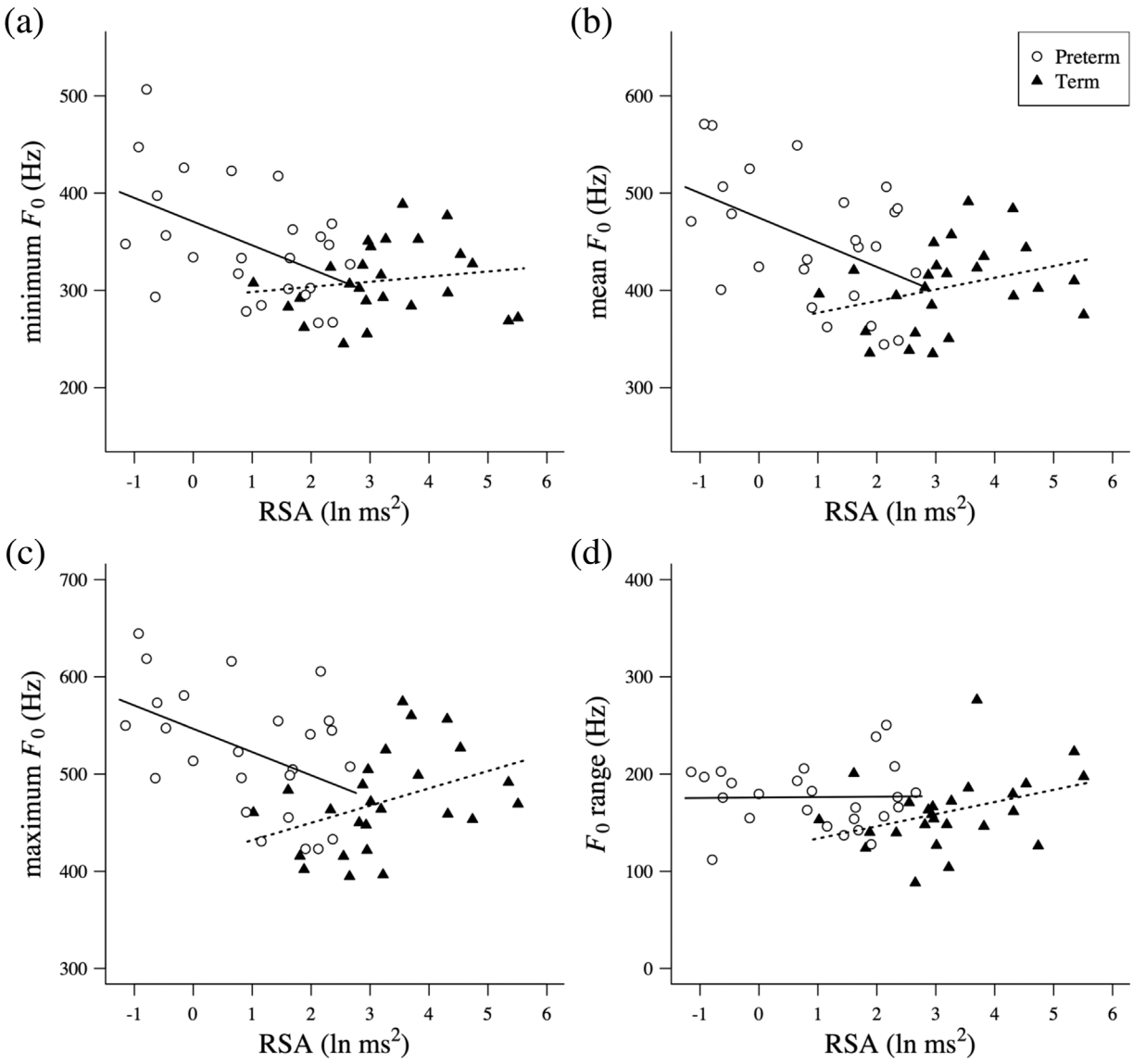

FIGURE 1 Scatter plots with regression lines showing relationships between RSA during quiet sleep and (a) minimum F_0 (preterm: $r = -0.49$, $p = 0.014$; term: $r = 0.16$, $p = 0.455$); (b) mean F_0 (preterm: $r = -0.46$, $p = 0.019$; term: $r = 0.31$, $p = 0.133$); (c) maximum F_0 (preterm: $r = -0.46$, $p = 0.022$; term: $r = 0.40$, $p = 0.051$); and (d) F_0 range (preterm: $r = 0.02$, $p = 0.933$; term: $r = 0.36$, $p = 0.075$) of spontaneous cries for preterm ($n = 25$, white circle) and term infants ($n = 25$, black triangle) at term-equivalent age.

right nucleus ambiguus is assumed to cause contracting laryngeal muscles and tightening vocal folds (Porter et al., 1988; Soltis, 2004; Stewart et al., 2013). Thus, the increased F_0 of spontaneous cries in preterm infants may be due to the hypertension of laryngeal muscles and vocal folds induced by lower vagal input in preterm infants. In addition, we found that the minimum F_0 was most associated with RSA among F_0 variables of spontaneous cries in preterm infants. Given that the minimum F_0 is thought to closely correspond to vocal fold size (Ey et al., 2007; Wermke & Robb., 2010), the tension of vocal folds induced by lower vagal function might most affect the increasing minimum F_0 of spontaneous crying.

On the other hand, we did not find negative correlations between RSA and F_0 of spontaneous cries in the term group, in contrast to previous observations (Porter et al., 1988). Term infants showed robustly higher RSA during quiet sleep than preterm infants. Thus, the relatively high vagal

Table 3. Summary of Hierarchical Multiple Regression Analyses for RSA Predicting F_0 Variable of Spontaneous Crying in Preterm Infants

	Minimum F_0			Mean F_0			Maximum F_0			F_0 range		
Predictor	β	*SE*	*t*	β	*SE*	*t*	β	*SE*	*t*	β	*SE*	*t*
Step 1												
Gestational age	0.13	0.23	0.56	0.19	0.23	0.82	0.23	0.23	0.96	0.20	0.23	0.39
Postmenstrual age	−0.39	0.26	−1.53	−0.31	0.26	−1.22	−0.25	0.26	−0.95	0.24	0.26	0.35
Weight at cry recording	0.41	0.28	1.48	0.36	0.28	1.29	0.26	0.28	0.90	0.27	0.28	0.35
	$\Delta R^2 = 0.12$, $\Delta F_{3,21} = 0.97$			$\Delta R^2 = 0.10$, $\Delta F_{3,21} = 0.53$			$\Delta R^2 = 0.08$, $\Delta F_{3,21} = 0.57$			$\Delta R^2 = 0.12$, $\Delta F_{3,21} = 0.94$		
Step 2												
RSA	−0.51	0.19	−2.63*	−0.46	0.20	−2.27*	−0.45	0.21	−2.15*	0.08	0.22	0.36
	$\Delta R^2 = 0.23$, $\Delta F_{4,20} = 6.93$*			$\Delta R^2 = 0.19$, $\Delta F_{4,20} = 5.16$*			$\Delta R^2 = 0.17$, $\Delta F_{4,20} = 4.61$*			$\Delta R^2 = 0.01$, $\Delta F_{4,20} = 0.13$		

Note: RSA, respiratory sinus arrhythmia; β, standardized regression coefficient; ΔR^2, chang value of *R* square; ΔF, change value of *F*-value.
*$p < 0.05$.

function in term infants might not cause hypertension of the vocal folds with regard to increased F_0 during spontaneous crying without any severe stress (e.g., a circumcision procedure; Porter et al., 1988). Nevertheless, RSA was also positively associated with the mean F_0, maximum F_0 and F_0 range of spontaneous cries in term infants. These positive associations might be explained by laxer vocal folds due to their relatively high vagal activity. An increase in vocal F_0 may also be associated with high subglottic pressure from the lungs (Titze & Ingo, 1994); specifically, the largest changes in F_0 due to subglottic pressure are likely to occur when the vocal folds are very short and lax (Titze, 1989). Hence, laxer vocal folds in term infants, due to their higher vagal activity, might be related to greater changes in F_0 (i.e., higher maximum F_0 and larger F_0 range).

Vocal F_0 is negatively related to body size, in particular vocal fold length (Titze & Ingo, 1994). However, we did not find a negative effect of weight on F_0 in preterm or term infants, consistent with our previous research (Shinya et al., 2014). Moreover, the negative relationships between birth weight and the F_0 of spontaneous cries were not strong, even in relatively large samples of term neonates (Wermke & Robb, 2010). Thus, it is possible that weight is not an appropriate measure of vocal fold size regarding the F_0 of infant crying, particularly in the same age class within species (Rendall, Kollias, Ney, & Lloyd, 2005). Future studies should also include an index of laryngeal size to explain individual differences in the F_0 of spontaneous cries that our results do not explain.

Our findings have several potential implications for clinical applications in preterm infants during the perinatal period. Because high-pitched cries are likely to induce greater physiological and psychological stress in caregivers (Crowe & Zeskind, 1992), the increased F_0 of spontaneous cries in preterm infants may contribute to their higher risk of child abuse and neglect (Friedman, Zahn-Waxler, & Radke-Yarrow, 1982; Spencer, Wallace, Sundrum, Bacchus, & Logan, 2006). Thus, early interventions to improve vagal function (e.g., kangaroo-care (Feldman & Eidelman,

Table 4. Summary of Hierarchical Multiple Regression Analyses for RSA Predicting F_0 Variable of Spontaneous Crying in Term Infants

	Minimum F_0			Mean F_0			Maximum F_0			F_0 Range		
Predictor	β	*SE*	*t*	β	*SE*	*t*	β	*SE*	*t*	β	*SE*	*t*
Step 1												
Gestational age	−0.10	0.21	−0.50	−0.24	0.21	−1.18	−0.22	0.21	−1.05	−0.19	0.21	−0.88
Weight at cry recording	−0.32	0.21	−1.57	−0.22	0.21	−1.08	−0.12	0.21	0.58	0.15	0.21	0.71
	$\Delta R^2 = 0.13$, $\Delta F_{2,22} = 1.70$			$\Delta R^2 = 0.14$, $\Delta F_{2,22} = 1.79$			$\Delta R^2 = 0.14$, $\Delta F_{2,22} = 2.17$			$\Delta R^2 = 0.04$, $\Delta F_{2,22} = 0.50$		
Step 2												
RSA	0.19	0.21	0.92	0.41	0.19	2.14*	0.51	0.19	2.66*	0.47	0.20	2.37*
	$\Delta R^2 = 0.03$, $\Delta F_{3,21} = 0.85$*			$\Delta R^2 = 0.15$, $\Delta F_{3,31} = 4.60$*			$\Delta R^2 = 0.23$, $\Delta F_{3,21} = 7.08$*			$\Delta R^2 = 0.20$, $\Delta F_{3,21} = 5.60$*		

Note: RSA, respiratory sinus arrhythmia; β, standardized regression coefficient; ΔR^2, chang value of *R* square; ΔF, change value of *F*-value.
*$p < 0.05$.

2003)) in preterm infants may be effective to moderate their higher-pitched cries and support parent-infant relationships. Moreover, neonatal vagal tone has been reported to be a predictor of better cognitive development in preterm infants (Doussard Roosevelt, Porges, Scanlon, Alemi, & Scanlon, 1997; Feldman & Eidelman, 2009). Considering adverse cognitive development in preterm infants (Aarnoudse-Moens, Weisglas-Kuperus, van Goudoever, & Oosterlaan, 2009), F_0 assessment of spontaneous crying may be useful as a supplementary means to assess vagal function, although RSA can be easily measured by non-invasive methods.

This study had several limitations. First, our preterm infants had a large variety of medical histories involving respiratory function (e.g., respiratory distress disease, neonatal apneic attack). However, we did not assess the effects of their respiratory function on RSA, although they did not need intubation for respiratory disease at term-equivalent age. Given that RSA is affected by respiratory rate and disease even in newborns (Henslee, Schechtman, Lee, & Harper, 1997; Patzak et al., 1996), our findings should be replicated in a larger, more controlled sample and should include an assessment of respiratory function. Furthermore, we measured HRV during quiet sleep for 5 min to assess vagal function, because short-term recording under controlled conditions is reportedly valid for assessing it (Longin, Schaible, Lenz, & König, 2005; Task Force, 1996). Nevertheless, the 5 min measures are too short to detect long-term trends in diurnal changes in HRV (e.g., very low frequency component) that have clinical importance (Malpas & Purdie, 1990; Massin, Maeyns, Withofs, Ravet & Gérard, 2000). Thus, further research is needed to investigate the effects of recording duration on the relationships between RSA and the F_0 of spontaneous crying.

In conclusion, we revealed the associations between RSA during quiet sleep and the F_0 of spontaneous crying in preterm and term infants at term-equivalent age. Lower RSA was related to higher overall F_0 in the preterm group; however, higher RSA was associated with a higher mean and maximum F_0, and a larger F_0 range in the term group. These results suggest that individual differences in vagal function may be associated with the F_0 of spontaneous cries via modulations of vocal fold tension in infants at an early developmental stage. However, it is still unclear whether these associations are limited to term-equivalent age or continue to a later developmental stage. Future longitudinal research is needed to clarify the developmental trajectory of the association between vagal function and the F_0 of spontaneous crying in preterm and term infants.

NOTES

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